# Isolation and characterization of polymorphic microsatellite *loci* in the razor clam *Solen marginatus*

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## Introduction

Microsatellites are highly polymorphic molecular markers, also referred as Simple Sequence Repeats (SSR), abundantly dispersed through most eukaryotic genomes. These are widely used in many fields ever since they were first described (Litt and Luty 1989). In recent years, they were used in aquaculture for linkage map contruction, population genetics and molecular evolution studies. However, only a limited number of microsatellites have been characterized in bivalves . In the present study we isolated novel microsatellite *loci* in *Solen marginatus* (Bivalvia, Solenidae) using two different approaches: construction of two enriched libraries for tri- and di-nucleotide repeat motifs and using Inter Simple Sequence Repeat (ISSR) markers (Fisher *et al.* 1996).

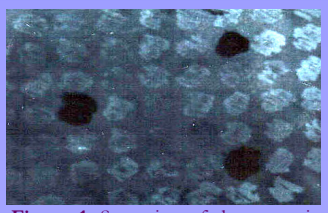

**Figure 1**. Screening of the genomic library hybridized with $(GAA)_7$.

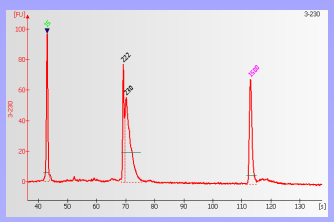

**Figure 2**. Image generated from the electropherogram showing two alleles of *locus* Sm230.

## Materials and methods

We used one population of *Solen marginatus* from Redondela (NW Spain). DNA extraction was carried out as in Winnepenninckx *et al.* (1993). *Loci* were isolated using both ISSR primers and two enriched libraries, $(GT)_8$ and $(CTT)_6$. For the construction of the enriched libraries we digested genomic DNA of an individual with the restriction enzyme RsaI, we hybridized the fragment with biotinylated repeat oligos, and then extracted the fragments of interest using streptavidin-coated magnetic beads. PCR amplification of ISSR markers was performed using the program Touchdown (Don *et al.* 1991), whereas microsatellite fragments were amplified following standard PCR. An initial screening of eight primer pairs (Figure 1) was evaluated for robust amplification and polymorphism using an Agilen 2100 Electrophoresis Bioanalyzer (Agilent Technologies) (Figure 2). Characteristics of the polymorphic *loci* were determined using GENEPOP (version 3.4), (Raymond and Rousset, 2004).

## Results and discussion

In this work we developed four microsatellite markers from the ISSRs and three from an enriched library of *Solen marginatus* (Table 1). The analysis of the ISSRs revealed that each sequence contained a microsatellite at the 3' and 5' ends of the insert, and many of them also had at internal sites (Figure 3). Isolation and characterization of these sequences obtained from ISSR markers constitute a feasible alternative to the construction of a genomic library.

We designed seven primer pairs for amplification of different microsatellite sequences. The polymorphism of these microsatellites was evaluated using 15 individuals of *S. marginatus*. *Locus* Sm221 was monomorphic. The *loci* Sm167, Sm187, Sm280 and Sm192 failed to amplify in all individuals, and *loci* Sm213 and Sm230 produced a clear polymorphic banding pattern. The number of alleles at each *locus* ranged from five to 10 and observed heterozygosity values ranged from 0.1667 to 0.9545. No *loci* showed significant linkage disequilibrium ($P<0.05$). These first three polymorphic microsatellite markers presented here could be useful for future studies on the population structure of this species.

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TGCTCTCTCT CTCTCTCTCT CTCGGGAAAT ATTTTTCTCT TGTATATTGA AATATACAGG   60
AGGAAAATAC CCCCCCCCCC ACACACACAC ACATCTTAGC AAGGTTTTGC ATGTGTGGTT  120
GGTACCTCGG TAGGAAGGTC AGTACCTCTG TTTGTATGTA AAATTTCCTA TCATAGCGAG  180
GTACTTTAGC CCAGAACCCT AACCCTAACC CTTACCCTAA CCCTAACCCT TTCCCATACC  240
CTTTCCCTGA ACATAACACC CAAATACACA AACCGAGGTA CCAAATTTGT ACCTCTTGAT  300
GTGATATAAA CCAACTCACA CACACGCCTG ACACACACAC ACACACACAC AGAGTCTGAT  360
TTTTTCTATC TTAGCGAGGT TTCGCACGTG TGGTTAGTAC CTCGGTAGGT AGTCTTGTAG  420
CTATGTTTGT ATGCAAAATG TCCTGTTATA GCGAGTTATT TTTTCCGAGC AATTTTTTTT  480
GTACCTCGGT ACGTAAATCA ATTTTGAAAG TATCCTGTTT TAGCCCAGAA CACTAACCCT  540
CACCCTAACC CATACCCTCT CCCTGACCCA AACACCAGAA AAAAACAATC CGAGGTACCA  600
GATTTGTACC TCTTCTGGTA GGTAAGAGAG AGAGAGAGAG AGCA 644
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**Figure 3**. ISSR fragment with microsatellites at the 3' and 5' ends, and three microsatellite sequences at internal sites.

**Table 1.** Microsatellites in *S. marginatus* developed from ISSR markers and enriched libraries.

| *Locus* | *Primer sequence (5'-3')* | *Motif* |
|---|---|---|
| Sm230 | F: ATTTGTATGCGTGCCTTTTGA<br>R: TCGTGGGGGGATAGAGTAACA | $(CTA)_7$ |
| Sm187 | F: TGAAATATACAGGAGGAAAATACCC<br>R: GGAAAGGGTTAGGGTTAGGGT | $(CA)_7$ |
| Sm213 | F: ACCCAAATACACAAACCGAGG<br>R: GCTCGGAAAAAATAACTCGCT | $(AC)_{10}$ |
| Sm221 | F: TATACCAGGACACACGCATTTCAT<br>R: AGTGCGTGACCGAAAAACCTT | $(TA)_5$ |
| Sm167 | F: ATTAATCATTAGCGCTGCG<br>R: GCGTGGACTAACGTCACTTTG | $(TG)_{19}$ |
| Sm280 | F: TCTTGCTTACGCGTGGACT<br>R: GGAAGACTGGTAGCTCTCT | $(CTT)_{31}$ |
| Sm192 | F: CTTAGCTCACGCTTAACACCAGA<br>R: CCTTGTGTGAACGAAATGTCTCA | $(CA)_7$ |

## References

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Litt M and Luty JA (1989) *Am J Hum Genet* 44: 397-401.

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